LIQUID-LIQUID EXTRACTION-GAS CHROMATOGRAPHY-ELECTRON CAPTURE DETECTION METHOD

INTRODUCTION

Different versions of the gas chromatographic (GC) method were used during the course of this disinfection by-product (DBP) occurrence project since the method was still undergoing major development during the utility sampling phase. A short description of the final version of the method will be given, followed by a history highlighting some of the major changes that occurred during the method development. The method changes improved the scope and quality of the method over the development period.

METHOD SUMMARY

The basic method used GC with a salted liquid-liquid extraction (LLE) procedure to quantitate and confirm 47 drinking water DBPs (Figure 1). For this method, two different GC columns were operated simultaneously (DB-1 and DB-5), which permitted the separation and quantitation for all of the analytes. The method included two different internal standards used as reference peaks. Samples were collected in two analytical fractions; however each fraction used the same sample preparation method. The two analytical fractions were used to accommodate the use of two different chemical preservatives (ascorbic acid and ammonium chloride). The method required two separate extractions and two GC injections of each sample to achieve the quantitation for all 47 DBPs. Sample preparation included collection of a 30 mL volume of sample, salting with 11 g of sodium sulfate and 1 g of copper sulfate, and extraction with 3 mL of methyl *tertiary*-butyl ether (MtBE). A mechanical platform shaker was used for automated sample extraction. The copper sulfate enhanced analyte recovery and aided in the extract transfer process. An autosampler injected sample extracts onto a split-splitless GC injection port, and a two-channel data system simultaneously collected the two chromatograms for each injection.

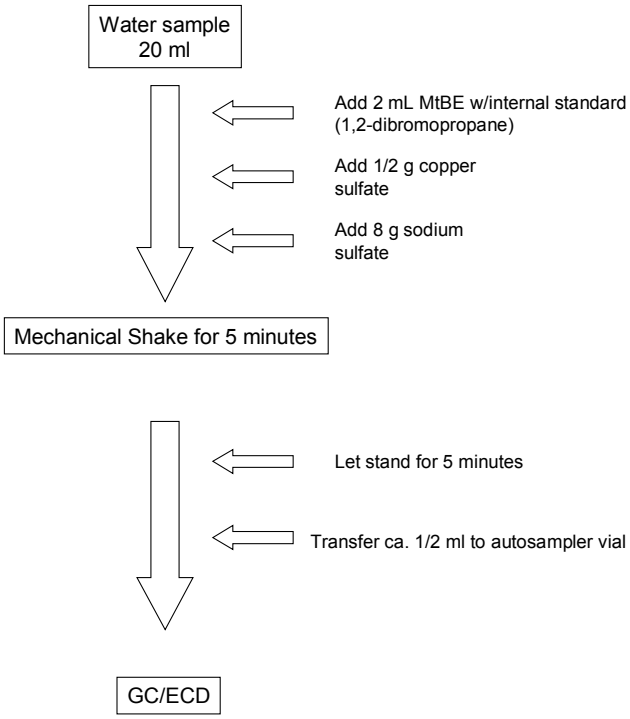

Figure 1. Summary of the LLE-GC-ECD method.

Sample Preparation

A 30 mL glass syringe was used to transfer samples into 40 mL glass vials. Daily procedural calibration standards were prepared with each set of samples using acidified reagent water. Sample matrix spikes and sample duplicates were prepared with each sample set. The MtBE extraction solvent contained two different internal standards. Because the MtBE was prepared with the internal standard, additional steps of adding the internal standard to each sample extract was eliminated. After 3 mL of MtBE was added, 11 g of dried sodium sulfate and 1 g of copper sulfate were added. The sample was capped and shaken briefly by hand before placing into a sample holder. After the solvent and salt were added to all of the samples, they were shaken using a vortex mixer for 11 min. A disposable Pasteur pipette was used to transfer approximately 2 mL of extract evenly between the two autosampler vials. One vial was stored in a freezer as a backup extract, and the other vial was was used for analysis.

Gas Chromatography Method

This GC method accomplished the separation and quantitation of 47 drinking water DBPs. The method involved the simultaneous analysis of one sample injection on two different analytical columns. The two different columns were attached to one injection port, allowing each sample to be analyzed by a GC equipped with two electron capture detectors (ECD). The two channels of data were collected simultaneously and processed sequentially. Unlike previous GC methods where one column is used as the primary analytical column for quantitation and a secondary column is used as a confirmation column, this method used both columns as primary analytical columns, with each column also used for confirmatory analysis. Using two different analytical columns allowed coeluting peaks to be resolved.

Two sets of samples collected from the each location because two different sample preservatives were required. Forty-one compounds were preserved and collected using ascorbic acid (AA). Ammonium chloride (AC) was used to preserve six other compounds (tri-halonitromethanes) that could not be preserved using ascorbic acid. Both the ammonium chloride and the ascorbic acid fractions were analyzed using the dual primary column analysis method. When analyzing the ascorbic acid fraction, one column could separate 25 compounds, and the other column could separate the other 16 compounds. Some compounds could be resolved on both columns, while other compounds could be resolved only on one of the columns. When a compound was separated on both columns, one column was used as the primary quantitation column, and the other column used for confirmation. Table 1 lists which DBPs coelute for each column. Ascorbic acid was used as a preservative for all sampling locations. Later in the study, ammonium chloride was used as a preservative for a smaller subset of those same sampling locations

When ammonium chloride-preserved samples were analyzed using the dual primary column analysis, 4 compounds could be resolved on one column, and the other two on the other column. Both columns were used for confirmation, as described for ascorbic acid-preserved samples. Ammonium chloride-preserved samples were extracted and analyzed using the same LLE procedure and GC conditions as for ascorbic acid-preserved samples.

Four separate GC software methods were developed to allow all 47 compounds to be analyzed. The 47 compounds were analyzed by producing four different chromatograms and calibrating most of the compounds twice. Data processing was done in pairs for each analytical fraction to enable cross-checking between columns. This aided in the analyte identification and detection process.

The primary column "A" was a DB-1 (J & W Scientific/Agilent, Folsom , CA, 30-m, 0.25- mm ID, 1-µm film thickness); primary column "B" was a DB-5 (J & W Scientific/Agilent, Folsom, CA, 30-m, 0.25-mm ID, 1-µm film thickness). Both analytical columns were installed onto a single GC injector (Model 3600, Varian Analytical Instruments, Walnut Creek, CA). The GC was equipped with two ECDs and an autosampler (Varian Analytical Instruments, Walnut Creek, CA). The autosampler injected 4.7 µL of extract onto a Model 1077 split-splitless injector operated in the splitless mode. The "A" and "B" channel ECD outputs were connected to a PE Nelson 970 interface (Perkin Elmer Corp., San Jose, CA).

Table 1. Gas chromatographic interferences for disinfection by-product analysis[a]

	DB-1 (10 coelutions)	**DB-5 (14 coelutions)**
1	cnm+bca+tca	can+tcan
2	tcnm+bdcan	Cnm+11dcp
3	1133tecp+13dbp	Bnm+bcan
4	dban+bdcnm	113tcp+tbcm
5	(ban+i)^	tba+dbim
6		bc+tban
7		1133tecp+cdim
	^ ban appears to coelute with an interference peak	
	Shouldered Peaks*	**Shouldered Peaks**
1	bnm & bcan & i	tca & dcan
2	13dcp & i	11dbp & dban & bcim
3	tbm & i	Bdim & i
4	bcim & 11dbp	
5	dbim & i	
6	111tbp & i	
	*i=unknown interference	

[a] Compound Abbreviations are Shown in Table 3

The GC operating conditions shown in Table 2 were optimized to enhance sensitivity. A low injection temperature of 87 °C was used to minimize degradation of thermally labile compounds. A large injection volume of 4.7 µL was chosen to increase sensitivity. Column flow rates and other conditions were adjusted to maximize resolution and detection for each compound.

Table 2. Gas chromatograph operating conditions

GC Temperature Program:											
	Temperature (°C):	35		139		301					
	Rate (°C/minute):		4		27						
	Time (minutes):	23		0		5					
Flow rates:	Helium carrier gas at 35°C		DB-1 Column = 2.3 ml/min								
			DB-5 Column = 1.3 ml/min								
	Head Pressure 14.3 psi										
Rear Injector Varian model 1077 capillary split/splitless											
	Split ratio = 12										
	Injector temperature = 87 °C										
	Injection mode splitless										
	Split valve program 0.89 min (relay=2)										
Detector Varian Nickel 63 Electron Capture Detector (ECD)											
	Two regular size ECD's (model # 02-001972-00)										
	Operating Temperature = 297 °C										
	Make-up gas Nitrogen at 29.3 mL/min										
	Autozero on										
	Range 10										
Varian model 8200 Autosampler											
	Injection Volume = 4.7 uL										
	Solvent plug size= 0.1 uL										
	Slow injection rate= 2.3 uL/sec										
	Upper air gap and lower air gap selected										
	Viscosity = 4										
	Resevoir pressure= 27psi										
	Resevoir solvent= MtBE										
Other Parameters											
	Thermal stabilize time=1.07 min										
	Column standby temperature= 117 °C										
	Column A and B installed:	A=J&W DB-1, 30 meter, 0.25 mm I.D., 1 micron film thickness									
		B=J&W DB-5, 30 meter, 0.25 mm I.D., 1 micron film thickness									
	GC= Varian model 3600										
	A central laboratory gas manifold system supplies nitrogen and helium gas										
	Dual channel data aquisition 1 volt input to model 970 PE Nelson Interface/Buffer										
	Chromatography Software PE Turbochrome Navigator (ver4.1) 1987-1995										

Calibration and Data Processing

Two sets of calibration standards were prepared from five different intermediate stock solutions (Table 3). The ascorbic acid spiking solutions contained the first 41 compounds (Table 3), and used 7 different concentration points (over the range of 0.1 – 80 µg/L) for the calibration curve. An additional high concentration point was added for THM analyses to enable the concentration range to extend to 120 µg/L (ppb). Ammonium chloride spiking solutions contained 6 compounds (trihalonitromethanes) (Table 3), and used 7 different concentration points (over the range of 0.5 – 20 µg/L) for the calibration curve. Calibration standards were prepared daily from stock solutions. Standards and blanks were prepared in pH-adjusted, distilled water (adjusted to 3.5 with concentrated sulfuric acid). Direct standards (non-extracted standards) were also prepared with each daily batch of extractions. Individual stock solutions were prepared on an annual basis, intermediate stock solutions were prepared quarterly, and spiking solutions were prepared bimonthly. All sample extracts and standard solutions were stored in the freezer at -11 °C.

Method Development Highlights

A short chronology of the major steps in the method development will be discussed. Each step is included because it has affected the type and quality of the project data. The variations in methods used over the project period can help to identify differences in the data over the utility sampling period.

The GC method development started in December 1998 and continued through the end of the utility sampling phase (April 2002). From February 1999 to August 2000, initial GC-ECD, purge-and-trap-GC/MS, and solid phase microextraction (SPME)-GC/MS methods were developed. In October 2000, due to operational problems with the Varian 3500A GC, two other GCs (a Varian 3500B and a Varian 3600) were configured for the dual column-GC-ECD analyses. Between February and March 2001, adjustments were made to the GC temperature program to achieve better separations. Higher quality-control spike concentrations of THM standards (50 ppb) were also made during this time. In March 2001, 9 additional compounds were added to the GC method (dichloronitromethane, bromochloronitromethane, tribromonitromethane, 1,1-dibromopropanone, 1-bromo-1,1-dichloropropanone, 1,1,1-tribromopropanone, 1,1,3-tribromopropanone, 1,1,1,3-tetrachloropropanone, and bromodichloroacetonitrile). Between May and July 2001, the extraction method was improved to increase the concentration factor and improve analyte recoveries. At this point, ammonium chloride was also introduced as a second preservation chemical, and the remaining 4 analytes were added to the method (tetrabromochloroethane, dibromochloronitromethane, bromodichloronitromethane, and chloronitromethane), for a total of 47 analytes. An additional internal standard (2-bromo-1-chloropropane) was also added to aid in analyte identification.

Table 4 shows the improved recoveries that were accomplished by the adjustments in the LLE-GC-ECD method. Table 5 shows the method reporting limits (MRLs) for the LLE-GC-ECD method compared to the SPE-GC/MS and P&T-GC/MS methods. In general the LLE-GC-ECD method reporting limits were the same or lower than other methods (Table 5).

Table 3. Stock standard calibration preparation

btl	#	Compounds		Stk Date	Stk ppm	Chk Date	Purity	Adj conc	uL in 1mL ACN	conc ppm
A		**100ppm THM & 551B mix**								
1	1	chloroform	tcm	11/28	2000		99+	2000	**50**	100.0
2	2	bromodichloromethane	bdcm		Supelco			ppm		
3	3	chlorodibromomethane	cdbm		4-8140u					
4	4	bromoform	tbm		MeOH					
1	5	Dichloroacetonitrile	dcan	11/28	2000		99+	2000	**50**	100.0
2	6	bromochloroacetonitrile	bcan		Supelco			ppm		
3	7	dibromoacetonitrile	dban		4-8046					
4	8	trichloroacetonitrile	tcan		acetone					
5	9	1,1-dichloropropanone	1,1-dcp		551b					
6	10	1,1,1-trichloropropanone	1,1,1-tcp		dbp					
7	11	chloropicrin	tcnm		mix					
1	12	1,1,2,2-tetrabromo-1-chloroethane	tebce	9/28	3700	6/29	78.7	2912	**35**	101.9
B		**100ppm Halomethane mix**								
1	13	Bromochloroiodomethane	bcim	4/6	3400	5/16	96.4%	3300	**32.0**	105.6
2	14	Dichloroiodomethane	dcim	4/6	2100	5/16	90.2%	1900	**54.0**	102.6
3	15	Dibromoiodomethane	dbim	4/6	3500	5/16	99.0%	3500	**28.6**	100.0
4	16	Chlorodiiodomethane	cdim	4/6	3900	5/17	68.3%	2650	**38.0**	100.7
5	17	Bromodiiodomethane	bdim	4/6	4800	5/16	93.8%	4500	**23.0**	103.5
6	18	Iodoform	tim	4/5	6900	5/17	99.0%	6900	**15.0**	103.5
7	19	Tribromochloromethane	tbcm	4/6	4200	5/16	94.9%	4000	**26.0**	104.0
C		**100ppm Halo(acetonitrile & acetaldehyde) mix**								
1	20	Bromoacetonitrile	ban	9/28	4400	5/10	99+%	4400	**23.0**	101.2
2	21	Chloroacetonitrile	can	4/5	2000	5/10	99+%	2000	**50.0**	100.0
3	22	Dichloroacetaldehyde	dca	4/6	4600	5/14	99.0%	4600	**24.0**	110.4
4	23	Bromochloroacetaldehyde	bca	7/17	2400	7/19	54.1%	1298	**78.0**	101.3
5	24	Tribromoacetaldehyde	tba	4/6	3200	5/14	99.0%	3200	**32.0**	102.4
6	25	chloral	tca	9/23	1000		99+%	1000	**100.0**	100.0
D		**100ppm Haloketone mix**								
1	26	Chloropropanone	cp	4/10	2100	5/14	98.0%	2050	**50.0**	102.5
2	27	1,3-Dichloropropanone	1,3-dcp	4/5	6500	5/14	99.0%	6500	**15.4**	100.1
3	28	1,1,3-Trichloropropanone	1,1,3-tcp	4/5	1900	5/15	99.6%	1900	**54.0**	102.6
4	29	1,1,3,3-Tetrachloropropanone	1,1,3,3-tecp	4/6	2000	5/15	99.0%	2400	**42.0**	100.8
5	30	1,1,1,3-Tetrachloropropanone	1,1,1,3-tecp	4/6	2200	5/15	92.4%	2050	**50.0**	102.5
6	31	1-Bromo1,1dichloropropanone	1-b1,1dcp	4/6	2700	5/15	77.6%	2100	**50.0**	105.0
7	32	1,1-Dibromopropanone	1,1-dbp	6/29	1800	5/15	94.0%	1700	**60.0**	102.0
8	33	1,1,1-Tribromopropanone	1,1,1-tbp	4/6	2600	5/15	97.0%	2500	**40.0**	100.0
9	34	1,1,3-Tribromopropanone	1,1,3-tbp	6/29	2200	5/15	97.6%	2150	**48.0**	103.2
10	35	1,1,3,3-Tetrabromopropanone	1,1,3,3-tebp	4/6	6400	5/15	99.0%	2000	**50.0**	100.0
E		**100ppm Halonitromethanes + bc mix**								
1	36	Chloronitromethane	cnm	9/28	5300	5/14	99.0%	5300	**19.0**	100.7
2	37	Bromonitromethane	bnm	4/5	3100	5/14	99.0%	3100	**34.0**	105.4
3	38	Dichloronitromethane	dcnm	4/5	2900	5/14	99.0%	2900	**36.0**	104.4
4	39	Bromochloronitromethane	bcnm	4/10	1950	5/14	97.4%	1900	**54.0**	102.6
5	40	Dibromonitromethane	dbnm	4/5	3600	5/14	97.1%	3500	**30.0**	105.0
6	41	Benzyl chloride	bc	4/5	2300	5/14	99+%	2300	**44.0**	101.2
F		**30ppm AC Mix**								
1	42	Bromopicrin	tbnm	4/5	3300	5/14	99.0%	3300	**9.1**	30.0
2	43	Tribromoacetonitrile	tban	4/6	3700	5/14	99.0%	3700	**8.1**	30.0
3	44	Bromodichloroacetonitrile	bdcan	4/6	2400	5/10	94.8%	2300	**13.1**	30.1
4	45	Dibromochloroacetonitrile	dbcan	4/10	3500	5/14	42.1%	1500	**20.0**	30.0
5	46	Bromodichloronitromethane	bdcnm	4/5	3800	5/14	99.0%	3800	**8.0**	30.4
6	47	Dibromochloronitromethane	dbcnm	4/5	4400	5/14	99.0%	4400	**6.9**	30.4

Table 4. Improved extraction method comparison showing increased compound recoveries

	tcm	can	cp	TCAN	DCAN	BDCM	tca	dcnm	BAN	bdcan	bnm	bcan	
A Method	107	516	137	2093	2022	1489	2690	418	7537	4115	2502	2793	
B Method	74	272	86	1065	1172	310	1674	176	2077	1598	506	526	
% Improved	**45**	**90**	**59**	**97**	**73**	**380**	**61**	**138**	**263**	**158**	**394**	**431**	
	dcim	bcnm	111tcp	13dcp	TBM	dban	dbcan	11dbp	dbnm	111dcbp	113tcp	tbcm	dbim
A Method	257	3388	2030	1159	291	2547	875	3876	2912	577	945	102	29
B Method	118	2082	632	427	173	1441	249	2911	1899	169	262	49	14
% Improved	**118**	**63**	**221**	**171**	**68**	**77**	**251**	**33**	**53**	**241**	**261**	**108**	**107**
	TBA	tban	BC	CDIM	1133tecp	1113tecp	tbnm	BDIM	111tbp	113tbp	tim	1133tebp	
A Method	294	1653	12	101	184	1868	2	265	184	1834	247	96	
B Method	129	621	6	47	73	431	1	107	71	1249	76	47	
% Improved	**128**	**166**	**100**	**115**	**152**	**333**	**100**	**148**	**159**	**47**	**225**	**104**	
A Method	Extract 30 mL sample with 3 mL MtBE + CuSO4+ Na2SO4 - 11 min shake												
B Method	Extract 20 mL sample with 4ml MtBE + Na2SO4 only - 5 min shake												

CONCLUSIONS

The GC method produced various levels of data quality, as it was developed throughout the sampling period. The GC method became more reliable and robust over the development period. The final method was capable of measuring the 47 DBP analytes in this study.

Table 5. Method reporting limit comparison of three analytical methods

			GC-LLE		SPE		P&T	
No.	Compounds	symbol	mrl	count	mrl	count	mrl	count
A	**100ppm THM & 551B mix**							
1	chloroform	tcm	0.5	1			0.2	1
2	bromodichloromethane	bdcm	0.1	2	0.5	1	0.2	2
3	chlorodibromomethane	dbcm	0.1	3	0.5	2	0.5	3
4	bromoform	tbm	0.1	4	2.5	3	0.5	4
1	Dichloroacetonitrile	dcan	0.1	5	5	4	0.2	5
2	bromochloroacetonitrile	bcan	0.1	6	0.5	5	1.0	6
3	dibromoacetonitrile	dban	0.1	7	.5	6		
4	trichloroacetonitrile	tcan	0.1	8	0.5	7		
5	1,1-dichloropropanone	1,1-dcp	0.1	9	1	8	0.5	7
6	1,1,1-trichloropropanone	1,1,1-tcp	0.1	10	1	9	0.5	8
7	chloropicrin	tcnm	0.1	11	0.5	10	1.0	9
1	1,1,2,2-tetrabromo-1-chloroethane	tebce	0.5	12	2.5	11		
B	**100ppm Halomethane mix**							
1	Bromochloroiodomethane	bcim	5.0	13	1	12	0.5	10
2	Dichloroiodomethane	dcim	0.5	14	1	13	0.5	11
3	Dibromoiodomethane	dbim	0.5	15	1	14	0.5	12
4	Chlorodiiodomethane	cdim	0.1	16	2.5	15	0.5	13
5	Bromodiiodomethane	bdim	0.5	17	5	16	0.5	14
6	Iodoform	tim	2.0	18	2.5	17		
7	Tribromochloromethane	tbcm	0.5	19	0.5	18		
C	**100ppm Halo(acetonitrile & acetaldehyde) mix**							
1	Bromoacetonitrile	ban	0.1	20	5	19	2.5	15
2	Chloroacetonitrile	can	0.1	21			0.2	16
3	Dichloroacetaldehyde	dca	0.5	22				
4	Bromochloroacetaldehyde	bca	0.5	23				
5	Tribromoacetaldehyde	tba	0.1	24	5	20		
6	chloral	tca	0.1	25				
D	**100ppm Haloketone mix**							
1	Chloropropanone	cp	0.1	26			0.5	17
2	1,3-Dichloropropanone	1,3-dcp	0.1	27	2.5	21		
3	1,1,3-Trichloropropanone	1,1,3-tcp	0.1	28	2.5	22		
4	1,1,3,3-Tetrachloropropanone	1,1,3,3-tecp	0.1	29	5	23		
5	1,1,1,3-Tetrachloropropanone	1,1,1,3-tecp	0.1	30	5	24		
6	1-Bromo1,1dichloropropanone	1-b1,1dcp	0.1	31	1	25		
7	1,1-Dibromopropanone	1,1-dbp	0.5	32	0.5	26	0.5	18
8	1,1,1-Tribromopropanone	1,1,1-tbp	0.1	33	5	27		
9	1,1,3-Tribromopropanone	1,1,3-tbp	0.1	34	5	28		
10	1,1,3,3-Tetrabromopropanone	1,1,3,3-tebp	0.5	35	5	29		
E	**100ppm Halonitromethanes + bc mix**							
1	Chloronitromethane	cnm		36			0.5	19
2	Bromonitromethane	bnm	0.1	37	2.5	30		
3	Dichloronitromethane	dcnm	0.1	38	0.25	31	0.5	20
4	Bromochloronitromethane	bcnm	0.1	39	2.5	32		
5	Dibromonitromethane	dbnm	0.1	40	0.5	33		
6	Benzyl chloride	bc	2.0	41	0.25	34	0.5	21
F	**30ppm AC Mix**							
1	Bromopicrin	tbnm	0.5	42				
2	Tribromoacetonitrile	tban	0.5	43				
3	Bromodichloroacetonitrile	bdcan	0.5	44				
4	Dibromochloroacetonitrile	dbcan	0.5	45				
5	Bromodichloronitromethane	bdcnm	0.5	46				
6	Dibromochloronitromethane	dbcnm	0.5	47				